42. (New) The method of Claim 40, wherein the control capture reagent is an anti-immunoglobulin antibody.

43. (New) The method of Claim 33, wherein the test sample is selected from the group consisting of: whole blood, plasma, serum, urine, cerebrospinal fluid, saliva, semen, vitreous fluid, or synovial fluid.

44. (New) The method of Claim 33, wherein the analyte of interest is selected from the group consisting of: myoglobin, CK-MB, troponin I, and PSA.

45. (New) The method of Claim 33, wherein in step (f) the fluid in the sample transports any contacted analyte-binding particles not bound to the sample capture reagent or to the control capture reagent by capillary action beyond the control capture zone into a wicking pad.

REMARKS

Claims 16-32, drawn to a nonelected invention(s), have been canceled without prejudice to Applicants' right to pursue these claims in a later Continuation or Divisional application.

Claims 2 and 3 have also been canceled. The substance of Claim 2 has been incorporated into Claim 1. Claims 33-45 have been added; Claim 33 is equivalent to Claim 1, except that the substance of canceled Claim 3 has been incorporated. Claims 34-45, which depend either directly or indirectly from Claim 33, are parallel to Claims 4-15.

Applicants' Invention

Applicants invention is drawn to methods for quantitatively measuring the amount of an analyte of interest in a fluid sample. The methods involve providing a membrane having an application point, a contact region comprising analyte-binding particles, a sample capture zone, and a control capture zone, where the contact region is between the application point and the sample capture zone, and the sample capture region is between the contact region and the control capture zone. In the assays, a fluid allows transport components of the assay by capillary action

through the contact region, to and through the sample capture zone and subsequently to and through the control capture zone. The methods utilize an internal control to compensate for variability in specific binding of assay components during the assay (intrinsic assay variability).

The amount of binding of analytes to particles, as well as the location of particles in relation to positions on the solid phase, is in flux because of the flow of fluid in the membrane. Variations in the structure of the solid phase reactants, such as porosity of the solid phase reactants, as well as variations in the viscosity of the fluid sample and other factors, can contribute to variability in specific binding of components of the assays (intrinsic assay variability). Intrinsic assay variability differs from sample concentration variability, in which the amount of analyte in the test sample can vary. The methods of the invention compensate for intrinsic assay variability, by taking into consideration the variations that result from the dynamic nature of the assays, and thereby allow more accurate determination of the amounts of analytes of interest in solutions.

Rejection of Claims under 35 U.S.C. 112, second paragraph

The Examiner maintained the rejection of Claims 1-15, as being indefinite. In particular, the Examiner questioned how the particles in part (c) of Claim 1 could be immobilized in the contact region, if they subsequently in step (d) are mobilized and migrate by capillary action. As indicated in the Specification (see, e.g., p. 12, line 9 *et seq.*; p. 14, line 1 *et seq.*) "immobilized" refers to particles that are coated on and/or permeated in the membrane. Part (c) of the claim has been amended to incorporate this language and specify that the particles are coated on and/or permeated in the membrane.

The Examiner also indicated that it was unclear regarding to what the control capture reagent was binding in part (e) of Claim 1. As indicated in the Specification (see, e.g., p. 14, line 11 *et seq.*), the control capture reagent reacts with the analyte binding particles, but does not interact with the analyte to be measured. Part (e) of the claim has been amended to incorporate this language and specify that the control capture reagent can react with analyte binding particles but does not interact with the analyte of interest.. In addition, part (d) of the Claim has been amended to clarify that the contacted analyte-binding particles may or may not have analyte of interest bound to analyte-binding agent on the analyte-binding particles, to clarify what is

included in "contacted analyte-binding particles". Support for this amendment is found, for example, at page 16, line 3 *et seq.*

The Examiner also indicated that it was unclear how the amount of corrected analyte-binding particles was determined. As described in detail in the Specification (see, e.g., p. 7, line 11 *et seq.)* the methods of the invention involve inclusion, within the assay, of an internal control that includes a control capture reagent that specifically binds to contacted analyte-binding particles which may or may not have analyte bound to them. The behavior of the analyte-binding particles with regard to the control capture reagent is used to compensate for the amount of variability in the reaction of the analyte-binding particles with the surfaces of the assay, and the amount of variability of the analyte-binding particles can be taken into consideration in a determination of the amount of analyte of interest. In some embodiments, for example, a corrected amount of analyte-binding particles can be determined by use of a ratio between 1) the amount of analyte-binding particles that are arrested in the sample capture zone, and 2) the amount of analyte-binding particles in the control capture zone; or use of a ratio between 1) the amount of analyte-binding particles that are arrested in the sample capture zone, and 2) the sum of the amount of analyte-binding particles in the control capture zone and the amount of analyte-binding particles that are arrested in the sample capture zone. Nevertheless, one of ordinary skill can easily identify other appropriate calculations that can be used to eliminate the variability in the specific binding component of the reaction. The amount of analyte of interest can then be calculated from the corrected amount of analyte-binding particles.

In order to expedite prosecution, Claim 1 has been amended to specify that the corrected amount can be determined as a ratio between the amount of analyte-binding particles that are arrested in the sample capture zone, and the amount of analyte-binding particles in the control capture zone. Claim 33, which parallels Claim 1, indicates that the corrected amount can be determined as a ratio between the amount of analyte-binding particles that are arrested in the sample capture zone, and the sum of the amount of analyte-binding particles in the control capture zone and the amount of analyte-binding particles that are arrested in the sample capture zone.

Rejection of Claims under 35 U.S.C. 102(e)

The Examiner rejected Claims 1-15 under 35 U.S.C. 102(e) as being anticipated by Kuo *et al.* (US Patent 6,436,721).

In order for a reference to anticipate claims, the reference must teach every aspect of the claimed invention either explicitly or impliedly (see M.P.E.P. § 2131).

Kuo *et al.* describe an immunochromatographic assay which is designed to compensate for variability in the concentration of the analyte in the sample that is tested, using a second analyte that serves as a normalizing factor. Kuo *et al.* utilize an assay with a contact zone (the first zone) containing a labeled analyte binding partner that binds to the analyte of interest; a second zone that contains either immobilized analyte or immobilized antibody to the analyte (provided that it is specific for an epitope of the analyte that is different from that to which the binding partner binds); a third zone which contains a means for capturing analyte/labeled specific binding partner complex; and a fourth zone that measures a second analyte (e.g., creatinine in urine) that differs from the analyte of interest and serves as an indication of the concentration of the first analyte in the test sample.

Kuo *et al.* state that the second zone can also contain immobilized analyte. The present invention does not contemplate utilizing immobilized analyte. Rather, the "sample capture reagent" of the present invention is a reagent that forms a binding pair with the analyte of interest, in that it specifically and preferentially binds to the analyte of interest.

For example, in one embodiment described by Kuo *et al.*, the second zone contains bound analyte, such that there is a competition of free analyte for the bound and the bound label is inversely proportional to the analyte concentration. In this embodiment, the third zone contains bound antibody that is specific for the analyte, such that the analyte-label complex not bound in the second zone is captured in the third zone, and is in direct proportion to the concentration. In this way the ratio of what is bound in the second zone, to what is bound in the third zone, is used to indicate the specific binding of the antigen in the assay. The ratio described by Kuo *et al.* thus serves to increase the sensitivity of the assay to the analyte, by measuring both of these regions, because the concentration of analyte increases, the binding at the second zone goes down and the binding at the third zone goes up. Nevertheless, while this may increase sensitivity, it does not correct for assay variability.

In a second embodiment described by Kuo *et al.*, the second zone contains antibody to the analyte, and the third zone has more antibody to the analyte. The binding at both regions would be of the analyte. Kuo *et al.* indicate that a ratio is also generated from the signal in these two regions. Because both capture antibodies are specific for the analyte, it cannot correct for intrinsic assay variability.

Furthermore, the concentration of the separate marker (in the fourth zone) in Kuo *et al.* is used in a ratio to correct for the sample concentration variability, that is, the concentration of the analyte of interest in the test sample in Kuo *et al.* The present invention does not utilize such a separate marker or zone. The separate marker of Kuo *et al.* is not used for correcting intrinsic assay variability, and, in fact, cannot be used to correct for intrinsic assay variability (which is separate and distinct from sample concentration variability) since the concentration of the separate marker is unknown. In contrast to the methods described by Kuo *et al.*, the methods of the invention utilize an internal control that is a known quantity: therefore any variation of its signal is due to assay variability.

In view of these considerations, Kuo *et al.* do not teach every aspect of the claimed invention, because they describe only methods that correct for sample concentration variability, and do not describe a method that corrects for intrinsic assay variability. Therefore, the claimed invention is not anticipated by Kuo *et al.*

CONCLUSION

In view the amendments and discussion presented above, the application in condition for allowance. Applicants' Attorney respectfully requests that the Examiner reconsider and withdraw all rejections.

If the Examiner believes that a telephone conversation would expedite prosecution of the application, the Examiner is invited to call Elizabeth W. Mata at (915) 845-3558 (Mountain time

zone). If Elizabeth W. Mata cannot be reached, the Examiner is invited to call David E. Brook at (978) 341-0036.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Twice Amended) A method for quantitatively measuring the amount of an analyte of interest in a fluid sample, comprising:

a) providing a membrane strip comprising an application point, a contact region, a sample capture zone and a control capture zone, wherein the contact region is between the application point and the sample capture zone and the sample capture zone is between the contact region and the control capture zone;

b) contacting the application point of the membrane strip with the fluid sample to be assayed for the analyte of interest;

c) maintaining the membrane strip under conditions which allow fluid to transport analyte of interest in the fluid sample by capillary action through the strip to and through the contact region, the contact region having a population of analyte-binding particles [immobilized] coated thereon and/or permeated therein, wherein the analyte-binding particles are coated with an analyte-binding agent;

d) further maintaining the membrane strip under conditions which allow analyte of interest, if present in the sample, to bind to analyte-binding particles, thereby generating contacted analyte-binding particles which may or may not have analyte of interest bound to analyte-binding agent on the analyte-binding particles; allow the fluid in the sample to mobilize and transport contacted analyte-binding particles by capillary action through the strip to and through the sample capture zone, the sample capture zone having a sample capture reagent immobilized thereon; and allow contacted analyte-binding particles to bind to the sample capture reagent;

e) further maintaining the membrane strip under conditions which allow the fluid in the sample to transport contacted analyte-binding particles by capillary action through the strip to and through the control capture zone, the control capture zone having a control capture reagent immobilized thereon, wherein the control capture reagent can

react with analyte-binding particles but does not interact with the analyte of interest; and allow contacted analyte-binding particles to bind to the control capture reagent;

f) further maintaining the membrane strip under conditions which allow the fluid in the sample to transport any contacted analyte-binding particles not bound to the sample capture reagent or to the control capture reagent by capillary action beyond the control capture zone;.

g) determining the amount of contacted analyte-binding particles in the sample capture zone and the amount of contacted analyte-binding particles in the control capture zone;

h) determining a corrected analyte-binding particle amount [from the amount of analyte-binding particles in the sample capture zone and the amount of analyte-binding particles in the control capture zone by compensating for the amount of variability in the reaction of the analyte-binding particles with the surfaces of the assay,

wherein the amount of analyte of interest in the fluid sample is directly related to the corrected analyte-binding particle amount], wherein the corrected analyte-binding particle amount is a ratio of the amount of analyte-binding particles in the sample capture zone, to the amount of analyte-binding particles in the control capture zone.